The Role of Benzoate in Anaerobic Degradation of Terephthalate

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The effects of acetate, benzoate, and periods without substrate on the anaerobic degradation of terephthalate (1,4-benzene-dicarboxylate) by a syntrophic methanogenic culture were studied. The culture had been enriched on terephthalate and was capable of benzoate degradation without a lag phase. When incubated with a mixture of benzoate and terephthalate, subsequent degradation with preference for benzoate was observed. Both benzoate and acetate inhibited the anaerobic degradation of terephthalate. The observed inhibition is partially irreversible, resulting in a decrease (or even a complete loss) of the terephthalate-degrading activity after complete degradation of benzoate or acetate. Irreversible inhibition was characteristic for terephthalate degradation only because the inhibition of benzoate degradation by acetate could well be described by reversible noncompetitive product inhibition. Terephthalate degradation was furthermore irreversibly inhibited by periods without substrate of only a few hours. The inhibition of terephthalate degradation due to periods without substrate could be overcome through incubation of the culture with a mixture of benzoate and terephthalate. In this case no influence of a period without substrate was observed. Based on these observations it is postulated that decarboxylation of terephthalate, resulting in the formation of benzoate, is strictly dependent on the concomitant fermentation of benzoate. In the presence of higher concentrations of benzoate, however, benzoate is the favored substrate over terephthalate, and the culture loses its ability to degrade terephthalate. In order to overcome the inhibition of terephthalate degradation by benzoate and acetate, a two-stage reactor system is suggested for the treatment of wastewater generated during terephthalic acid production.

With an annual production of 14.4 million tons in 1993, purified terephthalic acid (PTA) is among the top 50 chemicals manufactured in the world (23). PTA is used in the production of polyethylene terephthalate (PET) bottles, polyester films, and textile fibers. Production of PTA is based on the well-established process developed by the American Amoco group (1, 6). The process consists of two steps. In the first step, crude terephthalic acid (CTA) is produced through "wet oxidation" of *p*-xylene with air, and in the second step CTA is upgraded through hydrogenation of impurities during formation of PTA (1). During both steps, wastestreams are generated with a high level of organic contamination. The main components in these wastestreams are, in decreasing order of concentration, terephthalic acid, acetic acid, benzoic acid, and *para*-toluic acid (3, 18, 21).

Wastewater generated during the production of terephthalic acid is traditionally handled by aerobic treatment technologies (12). Due to the lower nutrient and energy requirements and lower surplus biomass production, anaerobic pretreatment may represent an attractive alternative for or contribution to conventional aerobic treatment. Therefore, several technological studies have been conducted to assess the feasibility of anaerobic pretreatment of terephthalic acid wastewater (11, 15, 18), and approximately 10 full-scale treatment systems are currently in operation or under construction (3, 16, 21). Results obtained during these studies indicate that most waste-

water constituents are biodegradable under methanogenic conditions (9) and are hardly toxic to methanogenic organisms (5, 11). However, the degradation rates found in anaerobic bioreactors are low (3, 11, 18), and lag phases prior to degradation of the phthalic acid isomers are long, ranging from 1 to 3 months in batch studies (9) to more than 1 year in full-scale reactors (3, 21).

In anaerobic bioreactors, organic compounds are converted into a mixture of methane and carbon dioxide in a complex network of several types of bacteria. These metabolic networks have been studied extensively for the anaerobic degradation of important agroindustrial wastewater constituents, such as volatile fatty acids and alcohols (24, 26). Combined with the development of high-rate anaerobic bioreactors, which have the ability to uncouple the solid retention time and the hydraulic retention time (13, 14), this knowledge contributed to the successful implementation of high-rate anaerobic bioreactors for the treatment of concentrated, noncomplex wastewaters. In contrast to these relatively noncomplex substrates, hardly any information is currently available about the kinetic properties of the methanogenic degradation of aromatic pollutants. Furthermore, the influence of rapidly degradable substrates on the anaerobic degradation of aromatic substrates is poorly documented. This lack of information seriously hampers the successful introduction and application of anaerobic treatment methods for the more-complex wastewaters, such as those generated in the petrochemical industries. With respect to PTA-wastewater, it has been shown that terephthalate and *para*-toluate are the rate-limiting substrates in anaerobic bioreactors (3, 11, 17, 21). Taking into account that terephthalate is the main polluting compound in PTA-wastewater, we focused our work on the anaerobic degradation of this compound. In a related paper (10), we describe the kinetic properties of the

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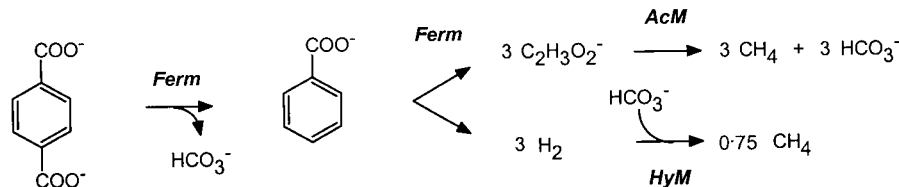
FIG. 1. Proposed degradation scheme for anaerobic degradation of terephthalate by a syntrophic culture consisting of terephthalate-decarboxylating and benzoate-fermenting organisms (Ferm), acetoclastic methanogens (AcM), and hydrogenotrophic methanogens (HyM).

different types of bacteria involved in the methanogenic degradation of terephthalate, as well as its *ortho*- and *meta*-oriented isomers. These experiments were conducted with enrichment cultures obtained from methanogenic granular sludge or digested sewage sludge. We postulated in that study that the anaerobic degradation of the phthalate isomers and benzoate proceeds according to the reaction scheme shown in Fig. 1.

The present study focuses on the following characteristics of a terephthalate-grown, methanogenic enrichment culture: (i) the influence of acetate and benzoate on terephthalate degradation, as well as the influence of acetate on benzoate degradation, and (ii) the influence of periods without substrate on benzoate and terephthalate degradation. Based on the results obtained, the specific role of benzoate in the anaerobic degradation of terephthalate is discussed, and the practical implications for anaerobic treatment of PTA-wastewater are presented.

MATERIALS AND METHODS

Biomass. The terephthalate-grown enrichment culture used in the experiments was obtained from granular biomass from a laboratory-scale anaerobic hybrid reactor as previously described (10). In order to cultivate a large amount of biomass, a continuously stirred 5-liter batch reactor was operated with the enrichment culture. The temperature of the polyacrylate reactor was controlled at $37 \pm 1°C$ by a thermostat bath circulator (Haake D1-L) connected to the double wall of the reactor. Prior to inoculation of the 5-liter cultivation reactor, cultures were transferred into serum bottles with increasing volume (up to 2 liters, liquid volume of 500 ml) in order to obtain a sufficient amount of biomass for inoculation. The cultivation reactor was operated in a fed-batch mode: approximately once a week, 1 liter of the culture was removed, and the reactor was replenished with a mixture of substrate and nutrients. By using this approach it was possible to grow a large amount of terephthalate-degrading biomass with a relatively constant volumetric conversion rate of 1 to 2 mM · day^{-1}. Due to the low growth rate of the terephthalate-degrading enrichment culture, it took approximately 6 months to obtain a stable culture in the 5-liter reactor.

Kinetic analyses. Experimental data were analyzed by using the previously described mathematical model, with the same set of experimentally determined or estimated parameter values for the different trophic groups in the terephthalate-degrading mixed culture (10).

Noncompetitive product inhibition of benzoate degradation by acetate was modelled by using the following rate equation for benzoate degradation:

$$R_{BA} = \frac{\mu_{BA}^{max}}{Y_{X_{Ferm}BA}} \cdot \frac{C_{BA}}{K_{BA} + C_{BA}} \cdot \frac{Ki_{C2}}{C_{C2} \cdot Ki_{C2}} \cdot C_{X_{Ferm}} \qquad (1)$$

where R, μ, Y, and C stand for volumetric conversion rate (mol · liter^{-1} · day^{-1}), maximum specific growth rate (day^{-1}), biomass yield (g · mol^{-1}), and concentration (mol · liter^{-1}), respectively. The subscripts X_{Ferm}, BA, and C2 stand for benzoate-fermenting biomass, benzoate, and acetate respectively. Ki_{C2} is a noncompetitive inhibition coefficient.

Online measurement of the CH₄ production rate. Methane production in the 5-liter cultivation reactor was measured through liquid displacement with a Mariotte bottle. Prior to the use of the Mariotte bottle, carbon dioxide was washed from the biogas by leading it over a 20% NaOH solution and a column filled with soda lime pellets for removal of water vapor and traces of carbon dioxide. The liquid displaced was collected in a container placed on a pressure sensor (DS-Europe model QB745) to detect the weight increase of the container. The pressure sensor was connected to a data logger (Campbell CR10), and weights were recorded every 30 min. The data logger was connected to a personal computer for continuous monitoring of the methane production.

Degradation of mixed substrates. In order to study the degradation of mixtures of two of the three substrates (acetate, benzoate, and/or terephthalate), batch experiments were performed in 300-ml serum bottles. Media were prepared with mixtures of substrates at the desired concentrations as described previously (10). All organic substrates were dosed as sodium salts from stock solutions and, if necessary, sodium concentrations were corrected through dosage of NaCl. Serum bottles were sealed with butyl rubber stoppers and aluminum screw caps. The headspace was flushed with a mixture of N_2 and CO_2 (70:30 [vol/vol]), and $Na_2S · 7$ to $9H_2O$ was dosed from a concentrated stock solution to obtain a final concentration of 150 mg · liter^{-1}. Serum bottles were preincubated at $37 \pm 1°C$ in an orbital-motion shaker and, after temperature equalization, inoculated by syringe with the terephthalate-grown enrichment culture. Samples for inoculation (10 to 20 ml) were taken from the cultivation reactor at the end of the exponential-growth phase. The total liquid volume in the serum bottles amounted to 50 to 70 ml. Throughout the experimental period, serum bottles were sampled at least once a day for analyses of the concentration of terephthalate and benzoate by high-pressure liquid chromatography, and volatile fatty acids, molecular hydrogen, and methane were analyzed by gas chromatography. A detailed description of the analytical methods applied can be found elsewhere (10). Measured concentrations in the headspace were corrected for the reduction in liquid volume due to sampling. All experiments were performed in duplicate.

Influence of periods without substrate on benzoate and terephthalate degradation. To study the influence of short periods without substrate on the terephthalate-degrading activity of the culture, 20-ml samples were regularly taken from the cultivation reactor. The samples were incubated with terephthalate (5 mM) in 117-ml serum bottles with a N_2-CO_2 gas-phase mixture for determination of the specific terephthalate-degrading activity of the biomass. The experimental procedure was basically the same as that described above, except no additional nutrients were included. Terephthalate degradation in the serum bottles was monitored by repeated measurement of the methane concentration in the headspace for 1 to 2 days. The volumetric terephthalate conversion rate (mol-terephthalate · l-inoculum^{-1} · day^{-1}) was calculated from the measured methane production by using linear regression techniques. Using this approach, the specific terephthalate-degrading activity, as measured at high terephthalate concentrations in the serum bottles, could be related to the terephthalate concentration and consequently the terephthalate conversion rate in the cultivation reactor.

In order to compare the influence of a short period of a few hours without substrate on the degradation of terephthalate and a benzoate-terephthalate mixture, a slightly different procedure was used. Serum bottles (117 ml) with a N_2-CO_2 gas-phase mixture were inoculated by syringe with a mixture of biomass and terephthalate from the cultivation reactor. Terephthalate, or a mixture of benzoate and terephthalate (final concentration of both substrates, 5 mM), was dosed to four bottles (in duplicate), while no substrate was dosed to four other bottles. After 1 day, benzoate or a benzoate-terephthalate mixture was dosed to the bottles that had not received any substrate at the moment of sampling. With this approach, the latter bottles were exposed to a period without substrate due to the depletion of the terephthalate in the inoculum. In time the concentrations of benzoate and terephthalate, the volatile fatty acids, and the methane content of the headspace were measured.

RESULTS

Mutual influence of acetate, benzoate, and terephthalate. The terephthalate-grown culture had the ability to degrade benzoate without a lag period, and specific conversion rates obtained with either benzoate or terephthalate as substrate were comparable (Fig. 2). When the culture was incubated with a mixture of terephthalate and benzoate, a sequential conversion of both substrates was obtained (Fig. 3). From this figure it can be seen that benzoate is the preferred substrate over terephthalate. Since hardly any terephthalate is degraded in the presence of benzoate, the degradation of a mixture of benzoate and terephthalate approaches diauxic degradation.

From Fig. 3 it can be seen that the calculated terephthalate conversion rate after complete removal of benzoate is 31% lower than the initial benzoate conversion rate, suggesting that
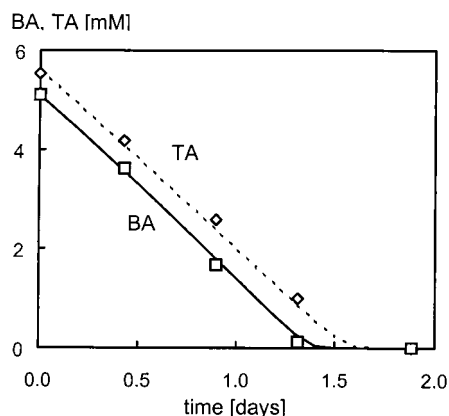
FIG. 2. Degradation of terephthalate (TA, ◇) or benzoate (BA, □) by the terephthalate-grown enrichment culture.

FIG. 4. Terephthalate (TA) degradation by the terephthalate-grown enrichment culture incubated with (◇) and without (□) 12 mM benzoate (BA). In the experiment in which the culture was incubated with a mixture of benzoate and terephthalate, benzoate was completely degraded after 6 days.

part of the terephthalate-degrading capacity is lost during the degradation of benzoate. At higher initial concentrations of benzoate and lower biomass concentrations, this loss in terephthalate-degrading activity is more pronounced, as shown in Fig. 4. Even though all of the benzoate is degraded within 6 days, a lag phase prior to terephthalate degradation of approximately 20 days is observed. Terephthalate conversion rates, after complete conversion of benzoate, are significantly lower compared to the experiment with terephthalate as the sole carbon and energy source.

A similar effect is observed in cultures incubated with a mixture of acetate and terephthalate (Fig. 5). Despite complete degradation of acetate within 7 days, no degradation of terephthalate is observed in a culture incubated with a mixture of terephthalate and acetate within 38 days.

The loss in degrading capacity is characteristic for terephthalate degradation because no loss in activity was observed when the terephthalate-grown culture was incubated with a mixture of benzoate and acetate (Fig. 6). Fully reversible product inhibition of benzoate degradation by acetate could accurately be described by a simple noncompetitive inhibition model (equation 1, above). For calculating the drawn lines in Fig. 6, a noncompetitive inhibition coefficient of acetate (Ki_{C2}) of
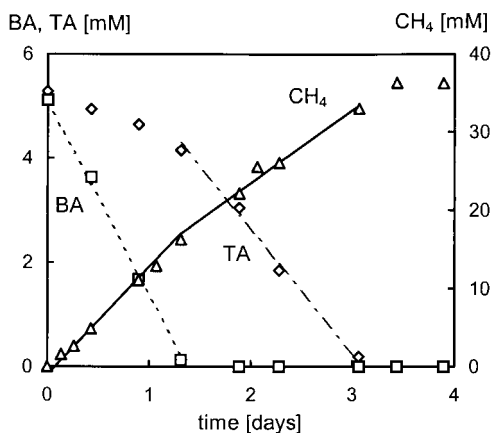
33 mM was used. It can furthermore be seen that at equal acetate concentrations, terephthalate degradation was completely inhibited, while benzoate conversion still proceeded.

Influence of substrate depletion on benzoate and terephthalate degradation. During cultivation of the terephthalate-grown enrichment culture, it was observed that if substrate was dosed after complete conversion of terephthalate, a long lag-phase (up to more than 1 month) occurred prior to terephthalate degradation. In order to quantify this inactivation due to substrate depletion, samples were regularly taken from the cultivation reactor and incubated with 5 mM terephthalate in 117-ml serum bottles. The specific terephthalate conversion rate was measured in the serum bottles for a period of 1 to 2 days. By this approach, the specific terephthalate-degrading activity of the culture was measured shortly before and after depletion of terephthalate in the cultivation reactor. From Fig. 7 it can be seen that the volumetric terephthalate conversion rate in the first sample, taken after approximately 2 days, is highly comparable to the rate in the 5-liter cultivation reactor.

FIG. 3. Degradation of a mixture of terephthalate (TA, ◇) and benzoate (BA, □) and concomitant methane production (CH₄, △) by the terephthalate-grown enrichment culture. Calculated lines indicate that the terephthalate degradation rate after complete removal of benzoate is approximately 31% lower than the initial benzoate degradation rate.
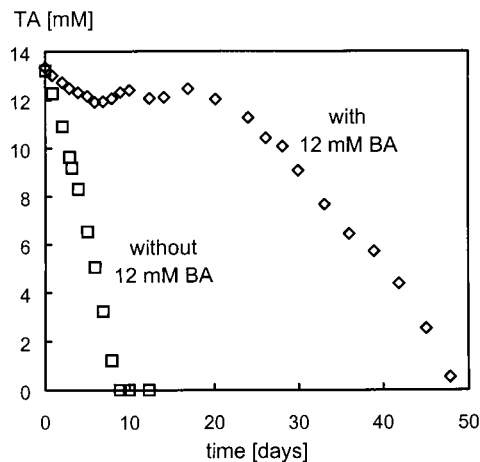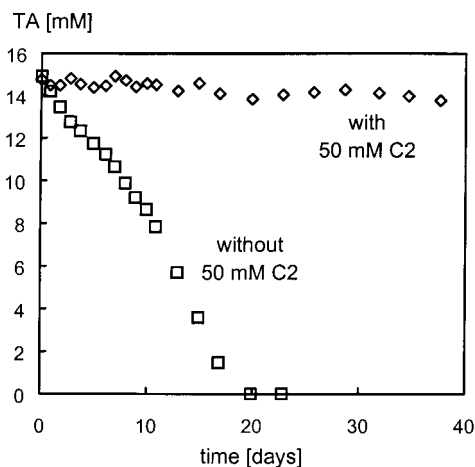

FIG. 5. Terephthalate (TA) degradation by the terephthalate-grown enrichment culture incubated with (◇) and without (□) 50 mM acetate (C2). In the experiment incubated with a mixture of terephthalate and acetate, the acetate was completely degraded after 8 days.
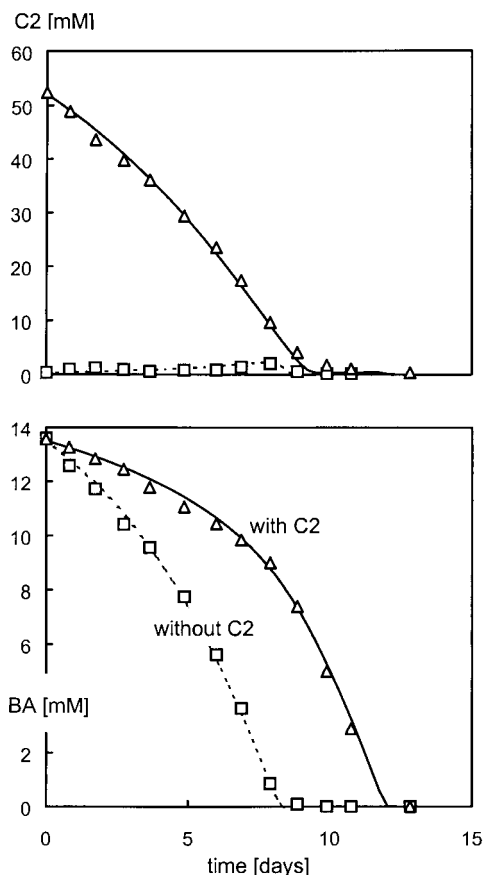
FIG. 6. Inhibition by acetate (C2, upper graph) of benzoate (BA, bottom graph) degradation by the terephthalate-grown enrichment culture. Markers correspond to measured concentrations, and drawn lines were calculated by using a noncompetitive inhibition model (equation 1) with a value for Ki_{C2} of 33 mM.

This result shows that no loss in activity occurred due to transfer of the culture.

In time the rate of methane production in the cultivation reactor decreases due to substrate depletion. Kinetically, this decrease in the methane production rate is expressed with the apparent half-saturation constant (K_{TA}), which was estimated to be 0.8 mM for terephthalate fermentation. From Fig. 7 it can be seen that the volumetric conversion rate of terephthalate degradation in the serum bottles (where sufficient substrate is present) proceeds parallel with the decrease in the conversion rate in the cultivation reactor. This observation evidently shows that the terephthalate-degrading culture almost completely loses its capacity to degrade terephthalate during short periods of starvation.

A similar experiment was performed with both terephthalate and a mixture of benzoate and terephthalate as substrates. The specific objective of this experiment was to determine if the culture only lost its ability to degrade terephthalate during short periods without substrate or if the benzoate-degrading activity was lost as well.

The data shown in Fig. 8 confirm that due to short periods without substrate, the initial terephthalate degradation rate is negligible during the first 2 days after terephthalate is dosed. Partial recovery of the terephthalate-degrading activity is obtained during the following days, but the terephthalate degradation rate remains distinctly lower than in experiments without a period of starvation. The initial increase in the methane

concentration observed in the serum bottles that received no substrate at time zero is due to the presence of terephthalate in the inoculum. From this initial increase in the methane concentration, the length of the period without substrate was estimated to be approximately 4 h.

The results are clearly different in experiments where a mixture of terephthalate and benzoate was incubated (Fig. 9). First of all, it can clearly be seen that the period without substrate hardly affects the conversion of benzoate. It is furthermore evident that terephthalate degradation is not affected by the period without substrate if a mixture of benzoate and terephthalate is used. These observations were confirmed by the highly parallel methane production curves.

DISCUSSION

Substrate competition during degradation of mixtures of benzoate and terephthalate. If benzoate and terephthalate are fermented by the same organism, the observed preference for benzoate degradation can be attributed to substrate competition. Fermentation of terephthalate is energetically more favorable than benzoate fermentation because decarboxylation of terephthalate is an exergonic process ($\Delta G^{0\prime} \approx -20$ kJ · mol^{-1}). Despite this energetic advantage of terephthalate fermentation, benzoate is the preferred substrate.

Based on the literature information (7, 8, 19, 20, 25), the initial steps in the degradation of terephthalate and benzoate
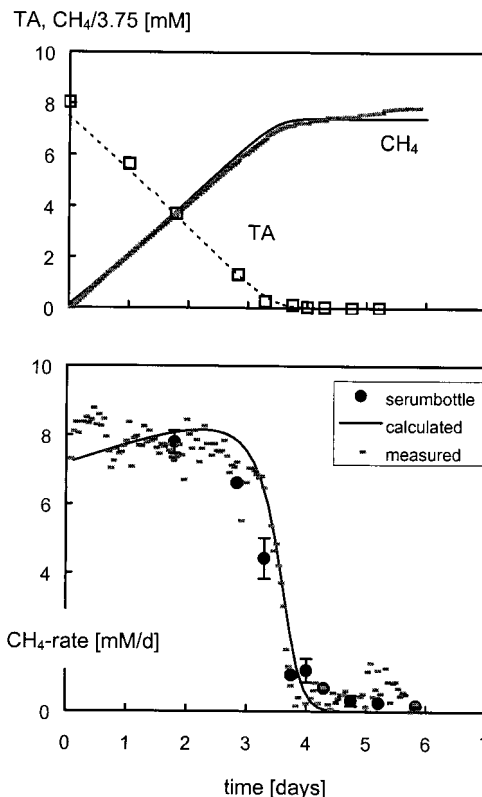

FIG. 7. Terephthalate (TA, □) degradation and cumulative methane production (CH_4^-) in the cultivation reactor (top graph) and the volumetric rates of methane production in the cultivation reactor ($^-$) compared to the volumetric terephthalate conversion rate of biomass sampled from the cultivation reactor and incubated with 5 mM terephthalate in serum bottles (●) (bottom graph). Lines showing terephthalate degradation and methane production in the cultivation reactor were calculated with a half-saturation constant for terephthalate fermentation (K_{TA}) of 0.8 mM.
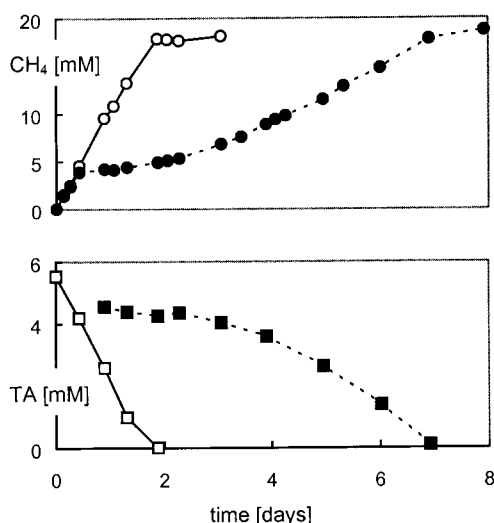
FIG. 8. Terephthalate degradation (TA, bottom graph) and concomitant methane production (CH$_4$, top graph) with (dashed lines, solid markers) and without (solid lines, open markers) a 4-h period without substrate.

likely proceed according to the pathway shown in Fig. 10. From this figure it can be seen that both terephthalate and benzoate degradation were proposed to converge at benzoyl coenzyme A (benzoyl-CoA), a central intermediate in the anaerobic degradation of aromatic compounds. We suggest that kinetic differences between these limited numbers of steps in the formation of benzoyl-CoA have to determine the preference for benzoate conversion because the preference for benzoate degradation over terephthalate is observed immediately after benzoate addition.

From the conversion steps shown in Fig. 10, the rate of substrate uptake across the microbial membrane may represent the rate-determining step in benzoyl-CoA formation. Since the pK$_a$ value for terephthalate (pK$_{a1,2}$ = 3.5) is lower than for benzoate (pK$_a$ = 4.2), the flux of terephthalate across the cytoplasmic membrane will be lower compared to benzoate, if both terephthalate and benzoate are activated at comparable rates. In the presence of both benzoate and terephthalate, this may result in higher concentrations of benzoyl-CoA from benzoate compared to terephthalate and, consequently, benzoate conversion will proceed faster. The activation rate may not be the rate-determining step in the overall conversion of benzoate and terephthalate, and therefore comparable maximum specific conversion rates for terephthalate and benzoate can still be obtained (Fig. 2). Aromatic ring reduction steps were proposed to be rate limiting in anaerobic benzoate degradation by *Rhodopseudomonas palustris* (22).

Product inhibition by acetate of benzoate and terephthalate degradation. Benzoate and terephthalate degradation are both inhibited by acetate. Benzoate inhibition by acetate could be well described with a noncompetitive inhibition model, with an inhibition constant (Ki$_{C2}$) of 33 mM. This value for Ki$_{C2}$ is in the same order of magnitude as the value of 40 mM determined in a defined coculture consisting of the benzoate degrader BZ-2 and *Methanospirillum* sp. strain PM-1 (2). In a coculture consisting of the benzoate degrader SB with *Desulfovibrio* sp. strain G-11, 50% inhibition of the benzoate degradation rate was obtained at an acetate concentration of approximately 10 mM (27).

In contrast to the inhibition of benzoate degradation by acetate, terephthalate degradation was found to be irreversibly
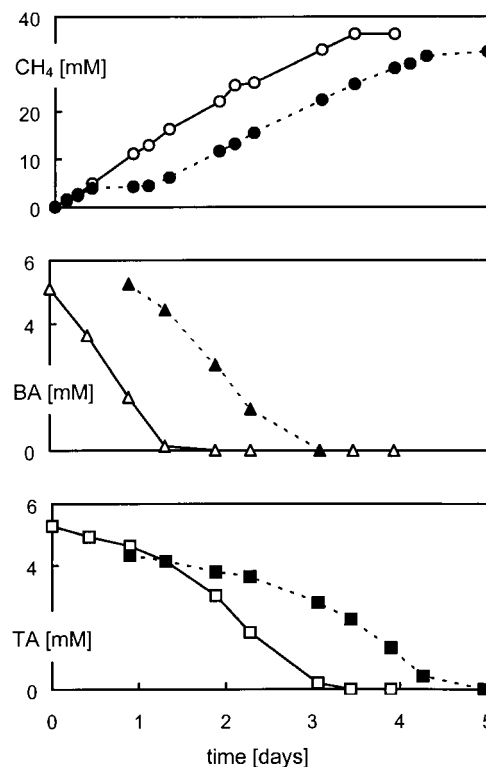

FIG. 9. Terephthalate (TA, bottom graph) and benzoate (BA, middle graph) degradation and concomitant production of methane (CH$_4$, top graph) by the terephthalate-grown enrichment culture incubated with a mixture of BA and TA with (dashed lines, solid markers) and without (solid lines, open markers) a 4-h period without substrate.

inhibited by acetate, resulting in long lag phases prior to terephthalate degradation after complete degradation of acetate (Fig. 5). The reasons for the apparent loss in terephthalate-degrading capacity are discussed below.

Deactivation of the terephthalate-degrading enrichment culture. The terephthalate-degrading enrichment culture lost a large part of its capacity to degrade terephthalate when (i) the culture had been incubated with a mixture of acetate and terephthalate, (ii) the culture had been degrading benzoate for a prolonged period of time (several days), or (iii) the culture had been exposed to a short period (hours) of starvation. The
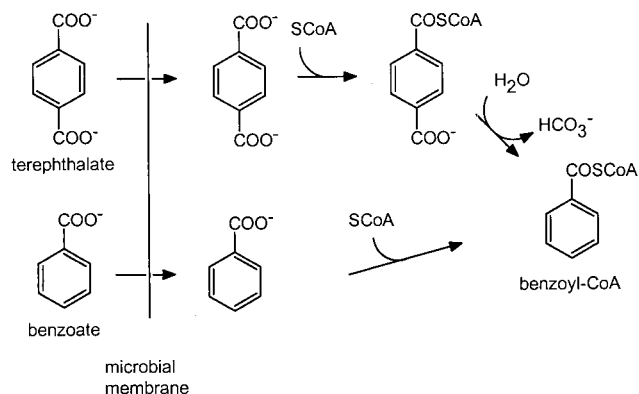

FIG. 10. Schematic representation of the proposed initial steps in terephthalate and benzoate degradation, both converging at benzoyl-CoA (19, 25).

extent of deactivation due to incubation with a mixture of benzoate and terephthalate appears to be related to the time needed for complete degradation of benzoate. At low biomass concentrations and/or high benzoate concentrations, long lag phases prior to terephthalate degradation were observed after complete removal of benzoate. At higher biomass and/or lower benzoate concentrations, the inhibition of terephthalate degradation by benzoate appeared to be partially reversible. Partially reversible inhibition of terephthalate degradation by benzoate has previously been observed with biomass from anaerobic bioreactors treating terephthalate-containing wastewater (5, 11). Irreversible inhibition of terephthalate degradation was observed in experiments incubated with a mixture of terephthalate and glucose (5). The inhibition in the latter case can probably be attributed to the accumulation of intermediates of glucose degradation (acetate and hydrogen).

Short periods without substrate resulted in an almost complete loss of the terephthalate-degrading capacity. As long as the periods without substrate were short (a few hours), part of the terephthalate-degrading activity could be recovered within a few days. However, when cultures were kept unfed for a period of several days, recovery of the terephthalate-degrading activity took more than 1 month (data not shown). The time periods without substrate leading to deactivation of the culture were too short to be explained by bacterial decay. Lag phases prior to growth due to periods without substrate have previously been reported for butyrate-degrading syntrophic cocultures (4).

The observation that the degradation of a mixture of benzoate and terephthalate is unaffected by a short period without substrate (Fig. 9) suggests that (one of) the first steps in the degradation pathway of terephthalate as shown in Fig. 10 are highly dependent on the latter steps (fermentation of benzoyl-CoA) in a coupled "chain reaction." It may be speculated that the organism needs the energy generated during fermentation of benzoyl-CoA (approximately 60 kJ/mol) to initiate the decarboxylation of terephthalate or to maintain gradients across the bacterial membrane, as may be needed for the active uptake of terephthalate. If the conversion of benzoyl-CoA is interrupted due to a feedless period, the chain is broken and one of the initial steps in terephthalate degradation may not be possible anymore.

In summary, it is suggested here that benzoate plays a peculiar double role in the degradation of terephthalate: benzoate (i) stimulates the degradation of terephthalate when supplied in a low concentration after a short period of starvation, (ii) inhibits terephthalate degradation when both substrates are present, and (iii) may cause a loss in terephthalate-degrading activity after benzoate degradation for a prolonged period of time.

Practical implications. The results described here have clear implications for anaerobic reactor technology for PTA-wastewater treatment. Due to the presence of both acetate and benzoate in the wastewater, the anaerobic degradation of terephthalate will be strongly inhibited in well-mixed reactors. Only if the reactor concentrations of acetate and benzoate can be kept low can growth on terephthalate be expected. Taking this into account, as well as the measured low growth rates on terephthalate of the methanogenic enrichment culture (10), we suggest that this type of wastewater should be treated in a staged bioreactor fashion. In the first stage of such a system, acetate and benzoate can be removed at high rates, while in the later stages terephthalate can be removed at lower volumetric conversion rates and maximized solid retention times. As a result of the preremoval of acetate and benzoate, anaerobic

mineralization of terephthalate in the latter stages can be optimized.

It should furthermore be emphasized that wastewater needs to be fed to the anaerobic bioreactors continuously in order to avoid inactivation of the terephthalate-degrading biomass. Since the industrial production of terephthalic acid is accomplished in a continuous process, continuous operation of the anaerobic reactors will normally not represent a problem. However, terephthalic acid production plants are normally stopped once or twice a year for a period of 1 to 2 weeks for maintenance purposes. It is clear that during these periods, measures should be taken to prevent a dramatic loss of the terephthalate-degrading capacity of the system due to feed interruption. If no sufficient measures are taken, a renewed startup procedure of several months may be required to regain the terephthalate-degrading capacity. If the terephthalate-degrading biomass in the latter stages of a staged anaerobic bioreactor is deactivated due to periods without substrate, it may be beneficial to direct a part of the benzoate-containing raw wastewater to the later stages of the process to enhance the recovery of the terephthalate-degrading activity.

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